

Pursuant to 37 C.F.R. §1.121, the applicant submits a clean set of claims as amended. The applicant has consolidated all separate amendments to the claims into a single clean version which is to be construed as a cancellation of all previous versions of the claims with respect to this application. The applicant respectfully requests entry of the clean version of the claims as set forth beginning on the next separate page:

- 51b
E1
1. A method of producing a mammal comprising the steps of:
- collecting sperm cells from a male of a species of mammal;
 - establishing an insemination sample having no more than 10% of the number of said sperm cells relative to a typical insemination sample;
 - inserting said insemination sample into a female of said species of said mammal;
 - fertilizing at least one egg within said female of said species of said mammal at success levels statistically comparable to a typical insemination dosage, wherein said steps of inserting said insemination sample into a female of said species of said mammal and fertilizing at least one egg within said female of said species of said mammal at success levels statistically comparable to the typical insemination dosage are each accomplished in a field environment; and
 - producing an offspring mammal.
- C1

2. A method of producing a mammal as described in claim 1 wherein said step of fertilizing at least one egg within said female of said species of said mammal at success levels statistically comparable to a typical insemination dosage comprises the step of fertilizing said at least one egg within said female of said species at success levels selected from the group consisting of at least 35%, at least 41%, at least 50%, and at least 90%.

51b
E1

3. A method of producing a mammal as described in claim 1 wherein said female of said species of said mammal has uterine horns and wherein said step of inserting said insemination sample into said female of said species of said mammal comprises the step of inserting said insemination sample both ipsi- and contra-lateral within the uterine horns of said female of said species of said mammal.

C2

4

4. A method of producing a mammal as described in claim 1 wherein said female of said species of said mammal has at least one uterine horn and wherein said step of inserting

C 2
5
7

said insemination sample into said female of said species of said mammal comprises the step of inserting said insemination sample deep within said uterine horn.

3

A method of producing a mammal as described in claim 1 wherein said step of inserting said insemination sample into a female of said species of said mammal further comprises the step of inserting said insemination sample deep within said uterine horns.

6

4

A method of producing a mammal as described in claim 6 wherein said step of inserting said insemination sample into a female of said species of said mammal further comprises the step of inserting said insemination sample within said uterine horn through the use of embryo transfer equipment.

7

5

A method of producing a mammal as described in claim 7 wherein said step of inserting said insemination sample into a female of said species of said mammal further comprises the step of inserting said insemination sample within said uterine horns through the use of embryo transfer equipment.

8

3

10.

A method of producing a mammal as described in claim 5 wherein said step of inserting said insemination sample into a female of said species of said mammal comprises the step of inserting said insemination sample twelve hours after the time which is generally regarded as optimal for a single insemination.

9 11.

7

A method of producing a mammal as described in claim 9 wherein said step of establishing an insemination sample comprises the step of establishing an unfrozen insemination sample, wherein said step of inserting said insemination sample into a female species of said mammal occurs not later than about seventeen hours from said step of establishing said insemination sample having no more than 10% of the number of said sperm cells relative to the typical artificial insemination dosage.

36

C

10 12

C 2

11 12 13

A method of producing a mammal as described in claim 9 wherein said step of establishing an insemination sample comprises the step of establishing an unfrozen insemination sample, wherein said step of inserting said insemination sample into a female species of said mammal occurs not later than about ten hours from said step of establishing said insemination sample having no more than 50% of the number of said sperm cells relative to the typical artificial insemination dosage.

A method of producing a mammal as described in claim 1 further comprising the step of determining a sex characteristic of a plurality of said sperm cells.

112

12 14

A method of producing a mammal having a predetermined sex as described in claim 13 further comprising the step of separating said sperm cells according to the determination of said sex characteristic.

13 15

12

A method of producing a mammal as described in claim 14 wherein said step of separating said sperm cells according to the determination of their sex characteristic comprises the steps of:

- establishing a sperm cell source which supplies sperm cells to be separated;
- sensing a property of said sperm cells;
- discriminating between said sperm cells having a desired sex characteristic; and
- collecting said sperm cells having the desired sex characteristic.

14

13

540 63

15

A method of producing a mammal as described in claim 15 wherein said steps of separating said sperm cells according to the determination of their sex characteristic further comprises the steps of:

- providing a flow cytometer;

37

C

- b. establishing a sheath fluid for said sperm cells; and
c. collecting said sperm cells having the desired sex characteristic.

12 15
14. A method of producing a mammal as described in claim 14 wherein collecting said sperm cells having the desired sex characteristic further comprises the step of cushioning said sperm cells from impact with a collector.

16
16. A method of producing a mammal as described in claim 16 wherein said step of providing a sorting flow cytometer comprises providing a high speed sorting flow cytometer, wherein said high speed flow cytometer sorts said sperm cells at a rate of at least 500 sorts per second.

17
17. A method of producing a mammal as described in claim 17 and further comprising the step of using an ovulatory pharmaceutical to cause multiple eggs to be produced.

18 20. A method of producing a mammal as described in claim 18 wherein said ovulatory pharmaceutical is injected in half day increments between any of days 2 and 18 of the estrus cycle.

19 21. A method of producing a mammal having a predetermined sex as described in claim 20 wherein said step of using an ovulatory pharmaceutical to cause multiple eggs to be produced comprises the step of injecting a dosage of follicle stimulating hormone.

20 22. A method of producing a mammal having a predetermined sex as described in claim 21 wherein said step of injecting said dosage of follicle stimulating hormone in approximately half day increments comprises a dosage level of 6, 6, 4, 4, 2, 2, 2, and 2 mg between days 9 and 12 inclusive of the estrus cycle and further comprising the step of injecting 25 mg and 12.5 mg of prostaglandin F-2-alpha on the sixth and seventh dosages, respectively, of said follicle stimulating hormone.

21
23.

14
A method of producing a mammal as described in claim 16 and wherein said step of determining the sex characteristic of a plurality of said sperm cells and said step of sorting said sperm cells according to the determination of their sex characteristic further comprises the step of staining sperm cells of a male mammal with at least about 38 micro-molar content of stain.

22
24.

14
A method of producing a mammal having a predetermined sex as described in claim 16 further comprising the step of chemically coordinating a sheath fluid environment for sperm cells which is coordinated with both pre-sort and post-sort sperm cell fluid environments.

23
25.

12 13 14 15 16
A method of producing a mammal as described in claim 1, 2, 14, 15, 16, 17 or 18 wherein collecting sperm cells from a male of a species of mammal comprises collecting said sperm cells from a male of a species selected from the group consisting of bovines, and equines.

24
26.

23
A method of producing a mammal as described in claim 25 wherein said step of chemically coordinating a sheath fluid to create a sheath fluid environment for said sperm cells which is coordinated with both a pre-sort and a post-sort cell fluid environments comprises the step of establishing a cell source which supplies bovine sperm cells and the step of establishing a sheath fluid which contains about 2.9% sodium citrate.

25
27.

23
A method of producing a mammal as described in claim 25 wherein said step of chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment comprises the step of establishing a cell source which supplies equine sperm cells and the step of establishing a sheath fluid which contains a hepes buffered medium.

268

A method of producing a mammal as described in claim 16¹⁴ wherein said step of collecting said sperm cells having the desired sex characteristic further comprises the step of avoiding impact of said sperm cells with said collector.

C2

27²⁹

A method of producing a mammal as described in claim 26²⁶ wherein said step of avoiding impact of said sperm cells with said collector comprises the step of providing a collection container having a diameter of at least fifteen millimeters.

28³³

A method of producing a mammal as described in claim 28²⁶ wherein said step of avoiding impact of said sperm cells with said collector comprises the step of providing a collection container having stream matched physical characteristics. ?

34

166

A method of producing a mammal as described in claim 16¹⁴ wherein said step of collecting said sperm cells having the desired sex characteristic further comprises the step of providing a citrate collection fluid containing about six percent egg yolk prior to commencing said step of collecting.

30³⁰
167

A method of producing a mammal as described in claim 18¹⁴ further comprises the step of operating said flow cytometer with in the range of about 5 kilohertz to about 50 kilohertz.

32 31

168

A method of producing a mammal as described in claim 165¹⁴ further comprises the step of sorting said sperm at a rate of at least 1200 sorts per second.

B8

32

169

A method of producing a mammal as described in claim 3¹ wherein said step of establishing a insemination sample having a low number of said sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an insemination sample selected from the group consisting of: a bovine insemination sample of no more than one hundred thousand sperm cells, a bovine insemination sample of no more than two hundred fifty thousand sperm cells, a bovine insemination sample of no

40

C

more than three hundred thousand sperm cells, an equine insemination sample of no more than one million sperm cells, an equine insemination sample of no more than five million sperm cells, an equine insemination sample of no more than ten million sperm cells, and an equine insemination sample of no more than twenty-five million sperm cells.

33
170. A method of producing a mammal as described in claim 1 wherein said steps of inserting
112 at least a portion of said insemination sample into a female species of said mammal and fertilizing at least one egg within said female species of said mammal at success levels statistically comparable to the typical unsexed artificial insemination dosage in a field environment comprises the steps of repetitively inserting a significant number of insemination samples into a significant number of ~~female species of said mammal~~ *of said female of said species* in rapid succession and in farm or ranch conditions.

134
171. A method of producing a mammal as described in claim 14 wherein said step of inserting
112 said insemination sample having said low number of said sperm cells into a female of said species of said mammal comprises inserting an insemination sample having a low number of sperm a substantial portion of which have the desired sex characteristic.

35
172. A method of producing a mammal as described in claim 189 wherein said step of inserting an insemination sample having a low number of sperm, wherein a substantial portion of said sperm have the desired sex characteristic comprises selecting said insemination sample having a low number of sperm from a group consisting of an insemination sample having a low number of sperm, wherein at least 60 percent of said sperm have the desired sex characteristic, an insemination sample having a low number of sperm, wherein at least 70 percent of said sperm have the desired sex characteristic, an insemination sample having a low number of sperm, wherein at least 80 percent of said sperm have the desired sex characteristic, and an insemination sample having a low

number of sperm, wherein at least 90 percent of said sperm have the desired sex characteristic.

- 136 35
172
173. A method of producing a mammal as described in claim 190 wherein said step of producing an offspring mammal comprises producing a predetermined sex ratio of fetuses.
- 03